The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

REMOVAL OF METHYL RED FROM AQUEOUS SOLUTION BY ADSORPTION ON TREATED BANANA PSEUDOSTEM FIBERS USING RESPONSE SURFACE METHOD (RSM)

(Penyingkiran Metil Merah daripada Larutan Akues oleh Serapan Fiber Batang Pisang Yang Telah Dirawat Mengunakan Kaedah Permukaan Sahutan (RSM))

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Abstract

The effect of adsorbent dose, pH and contact times on the removal of Methyl Red (MR) from aqueous solution by using agriculture waste (NaOH treated banana pseudostem fibers) were studied. The influence of these parameters on the removal of Methyl Red was examined by using a response surface method (RSM). The experiment was conducted by combining three parameters: adsorbent dose (500-1500 mg/L), pH (2-8) dan contact time (5-75 minutes). The Box-Behnken Design (BBD) in Response Surface Methodology (RSM) by Design Expert Version 8.0.4 (Stat Ease, USA) was used for designing the experiments. Results showed that the optimum conditions for removal of Methyl Red from an aqueous solution (100 mg/L) were as follows: adsorbent dose (1417.70 mg/L), pH (2.08) and contact time (42.94 minutes) with the value of a coefficient of determination, R^2 is 98.98 %. Meanwhile, the second-order polynomial equation indicates that pH and contact times mostly affect the removal of Methyl Red.

Keywords: response surface method (RSM), adsorption, methyl red

Abstrak

Dalam penyelidikan ini, kesan dos penyerap, pH larutan dan masa untuk membuang Methyl Merah dari larutan media menggunakan sisa buangan pertanian iaitu serat batang pisang yang telah dirawat dengan larutan Sodium Hydroxide (NaOH) telah dikaji. Kesan tiga faktor dalam pengurangan Methyl Merah telah dikaji menggunakan Kaedah Permukaan Sambutan (RSM). Eksperimen dijalankan dengan menggabungkan ketiga – tiga pemboleh ubah iaitu dos penyerap (500-1500 mg/L), pH larutan (2-8) dan masa tindakan (5-75 minit). Reka bentuk Box-Behnken (BBD) di dalam Kaedah Permukaan Sambutan (RSM) menerusi "Design Expert" versi 8.0.4 (Stat Ease, USA) telah digunakan untuk mereka bentuk eksperimen. Keadaan terbaik untuk membuang 100 mg/L Methyl Merah dari larutan media adalah seperti berikut: dos penjerapan (1417.70 mg/L), pH larutan (2.08) dan masa tindakan (42.94 minit) dengan nilai pekali penentu, R^2 sebanyak 98.98 %. Persamaan polinomial peringkat kedua diperolehi didapati pH larutan dan masa rawatan adalah paling memberi kesan terhadap pembuangan Methyl Merah dengan menggunakan fiber batang pisang yang telah dirawat.

Keywords: kaedah permukaan sambutan, serapan, metil merah

Introduction

In Malaysia, 97 % of the effluent discharged is mainly from three industrial categories, which are food industry, chemical industry and textiles industry [1]. Some of the industries used dyes in their daily processes such as textile, plastic, cosmetic, paper. Among of these various industries, textile ranks first in the usage of dyes for coloration of fiber [2]. Currently, it was estimated about 10,000 of different commercial dyes and pigments exist and over 7 x 10^5 tons are produced annually worldwide [3].

Organic dyes are an integral part of many industrial effluents and require a suitable method for disposal.The discharge of colored wastewater from the industries may present an eco-toxic hazard and introduce the potential danger of bioaccumulation, which may eventually affect human through the food chain [4]. However, these dyes, are not normally removed by conventional wastewater treatment systems. Hence, it becomes crucial to find out an alternative approach to removing colourant from the effluents before it can discharge into the water mainstream.

In recent years, adsorption has gained favor due to proven efficiency in the removal of pollutants from the effluents. Activated carbon has excellent adsorption efficiency for the organic compound and because of that; it is widely used [5]. But, it is getting expensive and many studies have been done to find low cost and effective of adsorbent to replace the activated carbon such as tea waste [6], coal ash [7], oil palm trunk fibre [8], sugar cane bagasse as well as rice husk, coconut shell and orange peel [9]. Meanwhile, in Malaysia, plant fibres from an agriculture sector often seen as a waste and not fully utilize. Data show; there are abundant sources of raw material in Malaysia that can be transformed into profitable, useable products [10]. Therefore, full utilization of natural plant for suitable application has become an attractive approach instead of letting them creating pollution problems.

In Malaysia, banana is the second most widely cultivated fruit. It is covering about 26,000 ha, with a total production of 530,000 metric tones [11], which mean much banana waste such as banana trunk fibers were obtained. This waste can be used to something much more valuable but has not received much attention. Scientifically, banana tree is called *Musa accuminata* that comes from *Musa* family. It was reported that the banana pseudostem fibers can be used as an enzyme immobilization matrix [12]. Therefore, it could be possible to utilize it as well in a different way of application such as for removal of azo dyes. Sodium hydroxide treatment has been identified to be able to improve the sorption capacity. The sorption capacity improvement occurred mainly by the dissociation of hydrogen ions bonded to the hydroxyl, carboxyl and other groups, which concomitantly offered a negatively charged surface [13].

The most classic and popular way to achieved highest removal efficiency is by the used of one variable at a time approach, but it involves a huge number of independent run and time consuming. Optimization also can be done by using Response Surface Methodology (RSM). RSM is a collection of mathematical and statistical techniques useful for modeling and analysis of problems in which the response of interest is influenced by several variables, and the objective is to optimize this response [14]. RSM proved very effective and time saving model for studying the influence of process parameters on the response (RSM) factor by significantly reducing the number of experiments and hence facilitating the optimum conditions [15].

In this research, the ability of banana trunk fiber as an alternative low-cost fiber for the removal of methyl red from the aqueous solution was investigated. The main objectives of this study are to obtain the optimum conditions for removal of azo dyes (Methyl Red) by using Response Surface Methodology approach. The sodium hydroxide treated banana pseudostem fibers were used as an adsorbent for this purpose.

Materials and Methods

Experimental Design

The Response surface method via Box-Behnken was employed to identify the relationship between a set of controllable experimental factors and observed results. This optimization process involves three major steps; performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model [16]. Table 1 shows the experimental range for each factor used and defined in three level (low, basal and high) coded as (-1, 0, +1).

Box-Behnken statistical designed with 3 factors, 3-level and 15 runs as shown in Table 2 and was used for the optimization procedure. This design consists of replicated center points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The three independent variables were coded according to the following equation (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad x_i = 1,2,3 \qquad (1)$$

where x_i is dimensionless value of an independent variable, X_i the actual value of an independent variable i, X_0 the actual value of the independent variable i at the center point and ΔX_i is the step change of X_i corresponding to a unit variation of the dimensionless value.

Table 1. Experimental values and coded levels of the independent variables

	Levels		
Independent variables	**-1**	**0**	**+1**
Adsorbent Dose, X_1	500	1000	1500
pH, X_2	2	5	8
Contact Time, X_3	5	40	75

Table 2. Box-Behnken experimental design matrix

	Coded values of the variables			**Actual values of the variables**		
Run	**Adsorbent dose (mg/L)**	**pH**	**Contact Time (min)**	**Adsorbent dose (mg/L)**	**pH**	**Contact Time (min)**
	X_1	X_2	X_3	X_1	X_2	X_3
1	0	+1	-1	1000	8	5
2	+1	0	-1	1500	5	5
3	0	0	0	1000	5	40
4	0	0	0	1000	5	40
5	0	-1	+1	1000	2	75
6	-1	-1	0	500	2	40
7	+1	0	+1	1500	5	75
8	+1	+1	0	1500	8	40
9	-1	0	+1	500	5	75
10	0	0	0	1000	5	40
11	0	+1	+1	1000	8	75
12	-1	0	-1	500	5	5
13	-1	+1	0	500	8	40
14	0	-1	-1	1000	2	5
15	+1	-1	0	1500	2	40

The mathematical relationship of the responses (% Removal) and the independent variables were modeled by a second order polynomial function as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

(2)

where Y is the predicted response, β_0, intercept, β_1, β_2 and β_3, linear coefficients, β_{11}, β_{22} and β_{33}, squared coefficients and β_{12}, β_{13} and β_{23}, the interactions coefficients of the equation and X_1, X_2 and X_3 are the independent variables.

The statistical analysis of the data was done by using Design-Expert 8.0.4 in order to determine the best model for the removal of Methyl Red (MR) from aqueous solution. An analysis of variance (ANOVA) by calculating F- value was employed. ANOVA was used to recognize the relationship between the response (% Removal) and the independent variables. The p-value was used as an indicator to verify the significance of each term. If the p-value was less than 0.05 indicates that the factor influences the experiment. The indicator for the fitness of the second order polynomial model was evaluated by coefficient of determination (R^2). In order to show the relationship and interaction between variables and the response graphically, the three dimensional surface plot or contour plots were employed.

Preparation of adsorbent and adsorbate

Banana pseudostem fibers from the family of *Musa acuminate balbisiana cultivars (BBBc)* or locally called as 'Pisang Nipah' were obtained from local farm at Taboh Naning, Alor Gajah, Melaka. The banana pseudostem fibers were cut into small pieces then washed using distilled water to remove any dirt before it was dried in drying oven at 65 °C for 24 hours. After that, it was ground using a grinder and sieved by using Endecotts Octagon Digital Sieve Shaker. The particles size obtained was in ranged of 63 – 500 micron. Then, 10g of pseudostem sample were stirred with 100 ml of 0.1M Sodium Hydroxide, NaOH solution for 1 hour at room temperature then filtered. The sample then washed using distilled water to neutral pH and dried in the oven at 55 °C for 24 hours. The resulting adsorbent was abbreviated as treated banana pseudostem fibers.

Methyl Red (MR), 2-[4-(dimethylamino)phenylazo] benzoic acid was used as an adsorbate for this study. Methyl Red is red in aqueous solution of pH under 4.4, yellow in pH over 6.2 and orange in between [17]. A stock solution of Methyl Red (100 mgL^{-1}) was prepared by dissolving 0.100 g of the dye in 1 L of distilled water. Different concentrations ranges between 20-100 mgL^{-1} were prepared from the stock solution. Distilled water was used for preparing all the solutions and reagents. The initial pH was adjusted with 0.1M HCl or 0.1M NaOH.

Batch Adsorption Studies

The effects of adsorbent dosage, pH and contact times were studied. The initial concentration was 100mg/L for all the samples. For the purpose of optimizing, the amount of the adsorbent was performed by adding different weight of pseudostem fiber in the range of 500 – 1500 mg/L into 50 ml of the Methyl Red solution. For each 50 ml of 100 mg/L, the pH was adjusted by using 0.1M HCl or 0.1M NaOH. The experiment was conducted at pH value range of 2-8. Then, the effect of treatment time was studied by varying the contact time in between 5 – 75 minutes at a constant speed of the stirrer. All the adsorption experiments were carried out at room temperature (27±2°C). All samples were analyzed using UV-VIS Spectrophotometer (Perkin Elmer UV/VIS Lambda EZ210) at λ_{max} of 463nm, to determine the final concentration of the solution. The percentage removal (Y) of Methyl Red dye by treated banana pseudostem fibers at time, t was calculated as:

$$Y = \frac{100(C_o - C_t)}{C_o} \quad (3)$$

where, C_o is the initial adsorbate concentration (mg/l), C_t is the adsorbant concentration (mg/l) at time t.

Analytical measurements

A series of standards solution with concentration ranging from 20 ppm to 100 ppm were prepared from Methyl Red (MR) stock solutions in 50 ml volumetric flask. Then the highest concentration (100 ppm) was used in wavelength scan application. The purpose of wavelength scan is to determine wavelength at maximum absorbance (λ_{max}) of methyl red. Then, the obtained λ_{max} was used in photometric scan. The prepared serial of dilutions above (known standards) were scan one by one and the absorbance readings were recorded. The standard calibration graph of methyl red standard was then constructed using a linear least square fit method. Photometric scan also were used to determine the absorbance of residual sample after treatment with banana fiber. The residual concentration after treatment; C_t can be calculated based on working 'standard curve' from a series of known standards (known concentration).

Results and Discussion

Statistical analysis

In order to study the combined effect of three factors, 15 experiments were performed in different combinations of the parameters (Table 3). Table 3 shows the predicted, and an experimental data related to percentage removals of Methyl Red obtained.

Model Fitting using RSM

Using the experimental results from Table 3, the full quadratic second order polynomial equation was fitted to the data appropriately and the equation was presented as follows:

$$Y = 33.97 + 1.65X_1 - 33.29X_2 - 4.36X_3 - 3.62X_1X_2 + 3.42X_1X_3 - 4.89X_2X_3$$
$$- 3.36X_1^2 + 29.397X_2^2 - 9.48X_3^2$$
(4)

The analysis of variance for the response has been summarized in Table 4. The large value of F indicates that most of the variation in the response can be explained by the regression equation. The p-value is used to estimate whether F is large enough to indicate statistical significance. If p values lower than 0.05 indicates that the model is statistically significant [18].

To decide about the adequacy of model to represent percentage of removal of methyl red, the adequacy of the model test were carried out and the results are given in Table 4. The p-value for the quadratic model was lower than 0.05 and the R^2 for the quadratic model was highest as compared with other model. Therefore, the quadratic model was chosen to illustrate the relationship between independent variable and the amount of methyl red removal.

Data obtained were also analyzed to check the normality of the residuals. The normal probability plot or dot diagram of the residuals is plotted as shown in Figure 1. From the plot, the data points lie reasonably close to the straight line, this shown that the developed model is adequate.

Table 5 shows the F-values and p value of the model for percentage removal of methyl red. The F value of the model is 54.10 and the p-value is <0.05, which showed that the model terms are significant. As shown in Table 5, the coefficient of determination (R^2) and adjusted R^2 of this model were 0.9898 and 0.9715, respectively. The coefficient of determination (R^2) is 0.9898 suggesting that 98.98% of the variability in percentage removal can be explained by this model. Regression also provides a way to evaluate the nature and the degree of correlation between dependent and independent variables. The closer the R^2 value is to 1.00, the stronger the model and the better the response predictions. The differences between values of R^2 (0.9898) and adj. R^2 (0.9715) are small; therefore, the similarity between R^2 and adjusted R^2 shows the adequacy of the model to predict the response.

The lack of fit F-value of 3.09 with p>0.05 implied that the lack of fit was not significant relative to the pure error. A non-significant lack of fit was considered good and was desired for the model to fit. Thus, a well-fitted model was obtained for the equation of percentage removal.

Table 3. Experimental and the predicted values yields for removing Methyl Red

Run	**Adsorbent dose (mg/L) (A)**	**pH (B)**	**Contact time (min) (C)**	**Y Experiment (% Removal)**	**Y Predicted (% Removal)**
1	0	+1	=1	24.78	29.85
2	+1	0	-1	25.14	23.72
3	0	0	0	31.51	33.97
4	0	0	0	37.91	33.97
5	0	-1	+1	92.77	87.70
6	-1	-1	0	84.38	88.02
7	+1	0	+1	19.28	21.84
8	+1	+1	0	28.39	24.75
9	-1	0	+1	10.27	11.35
10	0	0	0	32.5	33.97
11	0	+1	+1	10.27	11.7
12	-1	0	-1	29.82	27.26
13	-1	+1	0	31.2	28.69
14	0	-1	-1	87.73	86.65
15	0	-1	-1	5.00	3.65

Table 4. Adequacy of the model tested

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**Prob > F**	
Mean	27478.46	1	27478.46			
Linear	9038.32	3	3012.77	8.05	0.0041	
2FI	194.89	3	64.96	0.13	0.9380	
Quadratic	3788.64	3	1262.88	47.22	0.0004	Suggested
Cubic	109.98	3	36.66	3.09	0.2541	Aliased
Residual	23.74	2	11.87			
Total	40634.03	15	2708.94			

Source	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**	**PRESS**	
Linear	19.35	0.6869	0.6017	0.3492	8561.58	
2FI	22.14	0.7018	0.4782	-0.5060	19812.60	
Quadratic	5.17	0.9898	0.9715	0.8622	1813.12	Suggested
Cubic	3.45	0.9982	0.9874			Aliased

As shows in Table 5, the ANOVA analysis shows that the quadratic effects of pH (X_2) and contact time (X_3) were significant ($p<0.05$), while linear effect only pH was significant ($p<0.05$). The adsorbent dose does not give an effect to remove the Methyl Red. It may be because of the amount between high and low limit of the dose used is in small range. The pH give high significant to the removal of Methyl Red. This high significant may affected the low order interaction which is the adsorbent dose [18]. The removal of dyes by treated banana pseudostem fibers was found to be a maximum in the acidic medium.

The pH value of the dye solution plays an important role in the whole adsorption process and particularly on the adsorption capacity. As the pH of the adsorption solution was lowered, the positive charges on the surface increased. This would attract the negatively charged functional groups located on the reactive dyes [19]. Contact times also give an effect to the removal of Methyl Red where contact time increased, the percentage removal also increased.

Figure 2 shows the relationship between the actual and predicted values for adsorption of Methyl Red in aqueous solution by using the treated banana pseudostem fibers. The parity plot (Figure 2) shows the satisfactory correlation between the values of experimental and predictive values, wherein, the points cluster around the diagonal line which indicates the good fit of model.

Table 5. ANOVA of the second-order polynomial equation

Source	**Sum of squares**	**df**	**Mean Square**	**F Value**	**Prob > F**	**Remark**
Model	13021.85	9	1446.87	54.10	0.0002	significant
A-Adsorbent Dose(mg/L)	21.78	1	21.78	0.81	0.4082	
B-pH	8864.46	1	8864.46	331.46	< 0.0001	
C-Contact Time (min)	152.08	1	152.08	5.69	0.0628	
AB	52.49	1	52.49	1.96	0.2201	
AC	46.85	1	46.85	1.75	0.2429	
BC	95.55	1	95.55	3.57	0.1173	
A^2	41.76	1	41.76	1.56	0.2668	
B^2	3190.85	1	3190.85	119.31	0.0001	
C^2	332.03	1	332.03	12.42	0.0169	
Residual	133.72	5	26.74			
Lack of Fit	109.98	3	36.66	3.09	0.2541	not significant
Pure Error	23.74	2	11.87			

R^2 = 0.9898
Adjusted R-Squared = 0.9715

Effect of various parameters on Methyl Red dye removal efficiency

To evaluate the relationships and interactions of parameters, surface plots give good prescriptions (Figure 1 and 2). The behavior of the production conversion under the process conditions are presented in Figures 3 to 5. To study the effect of adsorbent dose and pH on Methyl Red removal, experiments were carried out by varying adsorbent dose from 500 to 1500 mg/L and pH from 2 to 8. The maximum adsorption of Methyl Red dye (Y) was 96.39% at low of pH (2.08) and higher of adsorbent dose (1417.70 mg/L) as shown in Figure 3. Therefore, there is an interaction between pH and adsorbent dose.The results also indicates that methyl red adsorption with treated banana pseudostem fibers mainly take place in acidic medium.

Figure 4 shows the effect of adsorbent dose and contact time on the removal of methyl red. In the studied range, time showed smallest effect with the maximum removal efficiency obtained was at 42.94 minutes. At 75 minutes, the percentage removal is lower due to an aggregation of dye molecules with the increase in contact time makes it almost impossible to diffuse deeper into the adsorbent structure at highest energy sites. As the mesopores get filled up and start offering resistance to diffusion of aggregated dye molecules in the adsorbents, that aggregation negates the influence of contact time [18].

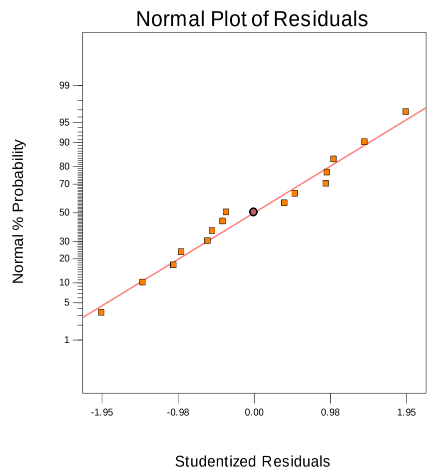

Figure 1. Normal % probability versus residual error of methyl red removal

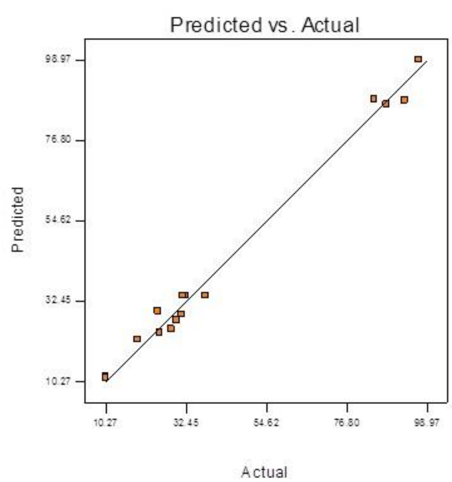

Figure 2. Scatter diagram of predicted response versus actual response of methyl red removal

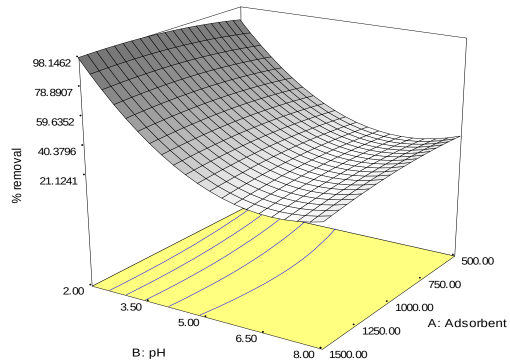

Figure 3. 3D response surface graph for dye removal versus adsorbent dose (mg/L) and pH for Methyl Red dye adsorption system

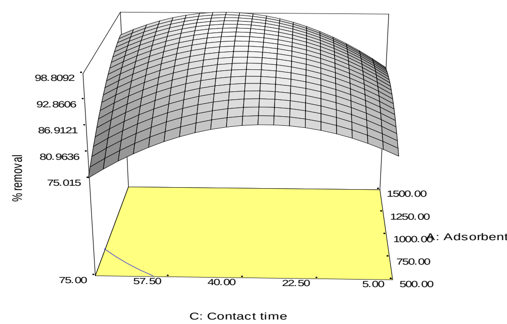

Figure 4. 3D response surface graph for dye removal versus adsorbent dose (mg/L) and contact time (min) for Methyl Red dye adsorption system

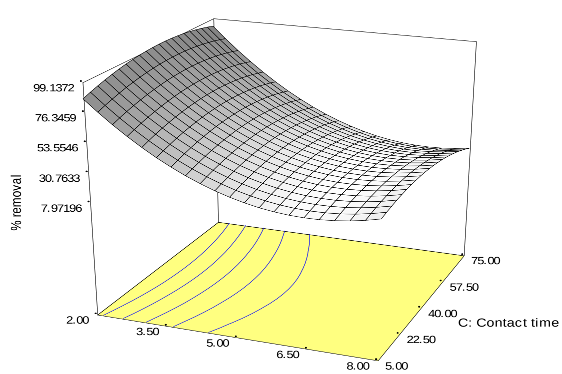

Figure 5. 3D response surface graph for dye removal versus pH and contact time (min) for Methyl Red dye adsorption system

Validation of the model

The maximum predicted adsorption capacity for optimum conditions in order to remove Methyl Red in aqueous solution was obtained through point prediction method and surface response plots is given in Table 6. The optimal level for adsorbent dose is 1417.70 mg/L at pH 2.08 and contact time 42.94 minutes with the maximum percentage removal is 96.39 %. The experiment was then performed at the optimal levels of the process parameters and the results shown in Table 7. The results are further analyzed using MINITAB 15 software package. One sample T-test was used to run the confirmation experiment's results. The P-value is 0.056 which is more than 0.05 (Table 8). This proven that, the results confirmed the validity of the model. Where the experimental data obtained was well represented by the Eq. (4).

Table 6. Optimum and confirmative values of process parameters for maximum removal efficiency

	Factor		
	Adsorbent Dose (mg/L)	**pH**	**Contact time (min)**
Optimal levels	1417.70	2.08	42.94

Table 7. Data of confirmation run

Run	**Adsorbance Dose (mg/L)**	**pH**	**Contact time (min)**	**Y (% Removal)**
1	1417.7	2.08	42.94	94.31
2	1417.7	2.08	42.94	93.10
3	1417.7	2.08	42.94	91.10
4	1417.7	2.08	42.94	95.55

Table 8. Result for validation

Variable	**N**	**Mean**	**St Dev**	**SE Mean**	**T**	**P**
Y (% removal)	3	93.515	1.895	0.948	-3.03	0.056

Conclusion

The optimum conditions for removal of methyl red from an aqueous solution by using banana fibers were successfully identified. RSM proved is a useful technique for studying the effect of several factors on responses by varying them simultaneously and carrying out a limited number of experiments. It was shown that a second-order polynomial regression model could properly interpret in the experimental data with a coefficient of determination (R^2) value of 98.98 %. Results also showed that the optimum conditions for removal of Methyl Red from an aqueous solution (100 mg/L) were as follows: adsorbent dose (1417.70 mg/L), pH (2.08) and contact time (42.94 minutes) with the value of maximum adsorption is 96.39%. Meanwhile, the equilibrium between the adsorbate in the solution and on the adsorbent surface was practically achieved in 42.94 minutes. A high similarity was observed between the predicted and experimental results, which reflected the accuracy and applicability of the model. Furthermore, this study shows that the Box-Behnken model is suitable to optimize the removal of methyl red. The treated banana pseudostem fiber has a good potential as an adsorbent for the removal of dyestuffs from aqueous solution.

References

1. Azira, S., Wong, T. N., Robiah, Y. and Chuah, T. G. (2004) Adsorption of methylene blue onto palm kernel shell activated carbon. E Proceeding. Regionalm Conference For Young Chemists 2004. Universiti Sains Malaysia, Penang, Malaysia.
2. Saiful Azhar S., Ghaniey Liew , Suhardy D., Farizul Hafiz K. , Irfan Hatim M.D (2005). Dye Removal from Aqueous Solution by using Adsorption on Treated Sugarcane Bagasse, *American Journal of Applied Sciences* 2 (11): 1499-1503, 2005.
3. Grag, V.K, M. Anita, R. Kumar and R. Gupta, 2004. Removal from simulated wastewater by adsorption using Indian Rosewood sawdust: A timber industry waste. *Dyes & Pigments*, 63: 243-250.
4. Atef S. Alzaydien (2009). Adsorption of Methylene Blue from Aqueous Solution onto a Low-Cost Natural Jordanian Tripoli. Science Publications. *American Journal of Environmental Sciences* 5 (3): 197-208.
5. Helen K.M., Iyyaswami R., Magesh G.P., and Lima R.M. (2009) Modelling, analysis and optimization of adsorpation parameter for H_3PO_4 activated rubber wood sawdust using response surface methodology (RSM). *Journal of Colloids and Surfaces B: Biointerfaces* 70: 35-45.
6. Amarasinghe B.M.W.P.K. and Williams R.A. (2007). Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater, *Chemical Engineering Journal* 132: 299.
7. Hawari A.H. and Mulligan C.N. (2006) Biosorption of lead(II), cadmium(II), copper(II) and nickel(II) by anaerobic granular biomass, *Bioresource Technology* 97: 692.
8. Hameed B.H., El-Khaiary M.I. (2008) Batch removal of malachite green from aqueous solutions by adsorption on oil palm trunk fibre: Equilibrium isotherms and kinetic studies, *Journal of Hazardous Materials* 154 : 237–244
9. Dhiraj S., Garima M., Kaur M.P. (2008) Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions – A review *Bioresource Technology* 99: 6017–6027.
10. Goh C.S., Tan K. T., Lee K. T., Bhatia S. (2010) Bio-ethanol from lignocellulose: Status, perspectives and challenges in Malaysia, *Bioresource Technology* 101: 4834–4841.
11. Mak Chai. Ho Y W., Liew K.W. and Asif J.M (2004). Biotechnology and in vitro mutagenesis for banana improvement. © 2004 Science Publications.
12. Rahim MZA (2009), Preliminary Study on Selected Malaysian Plant Based Material as New Immobilize Matrix, 2nd National Symposium on Fermentation Technology (NSFT 2009), Malacca International Trade Centre (MiTC), Ayer Keroh, Melaka, 14-15 .
13. Abdullah A.Z., Salamatinia B. and Kamaruddin A.H. (2009). Application of response surface methodology for the optimization of NaOH treatment on oil palm frond towards improvement in the sorption of heavy metals. *Desalination* 244 :227–238.
14. Montgomery, Douglas C. (2005). Design and Analysis of Experiments: Response surface method and designs. New Jersey: John Wiley and Sons, Inc.
15. Garg U.K., Kaur M.P., Sud D. and Garg V.K. (2009) Removal of hexavalent chromium from aqueous solution by adsorption on treated surgane bagase using response surface methodological approach. *Desalination* 249 : 475-479.
16. Annadurai G., Babu S.R., Nagarajan G. and Ragu K. (2000) Use of Box-Behnken design of experiments in the production of manganese peroxidase by Phanerochaete chrysosporium (MTCC 767) and decolorization of crystal violet. *Bioprocess engineering* 23: 715-719.
17. Mas Rosemal H. Mas Haris and Kathiresan Sathasivam (2009). The Removal of Methyl Red from Aqueous Solutions Using Banana Pseudostem Fibers. © 2009 Science Publications.

18. Paritosh Tripathi, Vimal Chandra Srivastava and Arvind Kumar (2009). Optimization of an azo dye batch adsorption parameters using Box–Behnken design. *Desalination* 249: 1273–1279.
19. Santhy K. and Selvapathy P. (2006). Removal of reactive dyes from wastewater by adsorption on coir pith activated carbon. *Bioresource Technology* 97 : 1329–1336.